Det Kgl. Danske Videnskabernes Selskab.
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BY

LOUIS SIGURD FRIDERICIA
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Some recent investigators have expressed doubts as to the retarding effect of vitamin A deficiency on the growth of bony structures. By measuring the length of the long leg bones of killed rats, some of which had been fed on adequate diet and some on diet deficient in vitamin A, E. J. QUINN, C. G. KING, and B. H. DIMIT (1929—30) and J. B. ORR and M. B. RICHARDS (1934) found that in the case of the vitamin A restricted animals these bones had increased in length relatively faster than the body had increased in weight. ORR and RICHARDS are of the opinion that the retardation of growth in vitamin A deficiency is a secondary consequence of the pathological conditions and of the diminished appetite in this avitaminosis and that no justification exists for assigning to vitamin A a specific influence on growth.

In order to investigate this problem in another way, we have measured the rate of growth of the incisors of two groups of albino rats, one of which was fed on an adequate diet and the other on diets deprived of vitamin A.

JOHN S. MARSHALL (1921) divided the growth process of the growing permanent incisors of rodentia into three stages, interstitial, extrusive, and curvative. The first deals with the formation of the concentric layers of dentine and, according to the above-mentioned writer, increases the thickness of the dentine stratum of rat teeth, on an average, about 0.01 mm. daily. The second deals with the extrusion,

or pushing out, of the tooth and is found by William H. F. Addison and J. L. Appleton (1915) to have an average rate of growth of 2.2 mm. per week for the upper, and 2.8 mm. per week for the lower, incisors of adult rats. The third deals with the curving of the incisors, which is caused by the more rapid growth of the labial side than of the lingual side of these teeth, the difference, according to Marshall, being an average of 0.7 mm. per week in rats.

The influence of diet on the extrusive growth of the incisors of rats has been examined by some investigators. Addison and Appleton (1915) found the same rate during feeding with hard food as during feeding with soft. B. Orban (1927) and William G. Downs (1931) varied the amounts of protein, fat, carbohydrates, and salts, in the diet, or gave a basal diet of wheat with the addition of various kinds of nutritive stuffs. H. J. Sedwick and B. S. Bibby (1933) examined the effect of pregnancy and of different diets. Fritz Egger (1925) found a slower rate of growth of the incisors with "completely vitamin free diet". Gilbert Dalldorf and Celia Fall (1930) experimented with guinea-pigs and found a deficiency of vitamin C to have a decidedly retarding influence on the rate of growth of the incisors.

There are no papers on the effect of vitamin A deficiency on tooth growth, but S. B. Wolbach and P. R. Howe (1925) mention that the rate of growth of the incisors of rats fed on a diet deficient in vitamin A was much slower than in rats fed on high protein diets, or diets deficient in the vitamin B complex.

In our experiments the extrusive growth of the lower incisors of albino rats has been measured.

Methods.

The extrusive growth of the incisors of rats can be ascertained either by clipping the exposed portion of one of these teeth every fifth day and measuring the length of the fragments (DALLDORF and FALL (1930)), or by marking the enamel and measuring the increase in distance between the mark and the gingival margin at certain intervals (as has been done by most other investigators). The two methods give different results, because fractured teeth grow faster than unfractured (ADDISON and APPLETON (1915), A. WILTON (1931), own measurements).

In our experiments we have made a fine transverse notch near the gingival margin on the labial side of one of the incisors and measured the distance between the mark and the margin once, or twice, weekly by means of a caliper gauge constructed especially for this purpose. The caliper gauge has two parallel needles which can be adjusted by a fine screw though still remaining parallel to one another. The head of the screw has a scale on which the distance between the needle points is read to an accuracy of 0.1 mm. During measurement, the mouth of the rat is kept open by introducing a finger into the diastema between the incisors and the molars.

The albino rats were of the laboratory stock, and were given the attention, feeding, and weighing, which is usual in vitamin experiments in this laboratory. Each cage contains one rat and is raised on legs in order to prevent coprophagy.

Series I. Adult rats on an adequate diet.

The standard error in estimations of the weekly extrusive rate of growth of the incisors of rats is not indicated

in the existing records. The adequate diet given in our experiments was:— Casein, 18 %. Rice starch, 54 %. Dried autolyzed brewer's yeast, 5 %. Butter fat, 15 %. Agar, 3 %. McCollum's salt mixture, No. 185, 5 %.

Table I. Extrusive growth of the incisors of rats, fed on an adequate diet.

	Weight	Average extrusive incisor growth per week	Standard deviation σ	Largest observed +deviation	Largest observed −deviation (in multiples of the standard deviation σ)
13 adult male rats ...	236—310 g.	2.65 mm.	0.22	$2.0 \cdot \sigma$	$2.5 \cdot \sigma$
12 — female — ...	152—226 g.	2.73 mm.	0.37	$2.1 \cdot \sigma$	$1.4 \cdot \sigma$
25 —	152—310 g.	2.69 mm.	0.30	$3.0 \cdot \sigma$	$2.0 \cdot \sigma$
9 young rats	47—272 g.	3.35 mm.	0.47	$3.1 \cdot \sigma$	$1.8 \cdot \sigma$

The weekly rate of growth of the incisors was measured in 25 adult rats, 13 males and 12 females, for 2—4 weeks. The results are seen in Table I. The average rate of growth was 2.7 mm. per week, with a standard deviation of 0.3 mm. for a single measurement. The difference between the average rates for males and females is 0.08 mm. As this is only 1.6 times the standard deviation in the 32—38 measurements for each sex respectively ($\sigma = 0.05$), the difference is without significance.

Series II. Young rats on an adequate diet.

It is not known whether the rate of the growth of the incisors is the same in young as in adult rats. As it is necessary to use young rats for experiments comparing changes in tooth growth, which are of dietary origin, with changes in the increase in weight, this matter must be cleared up.

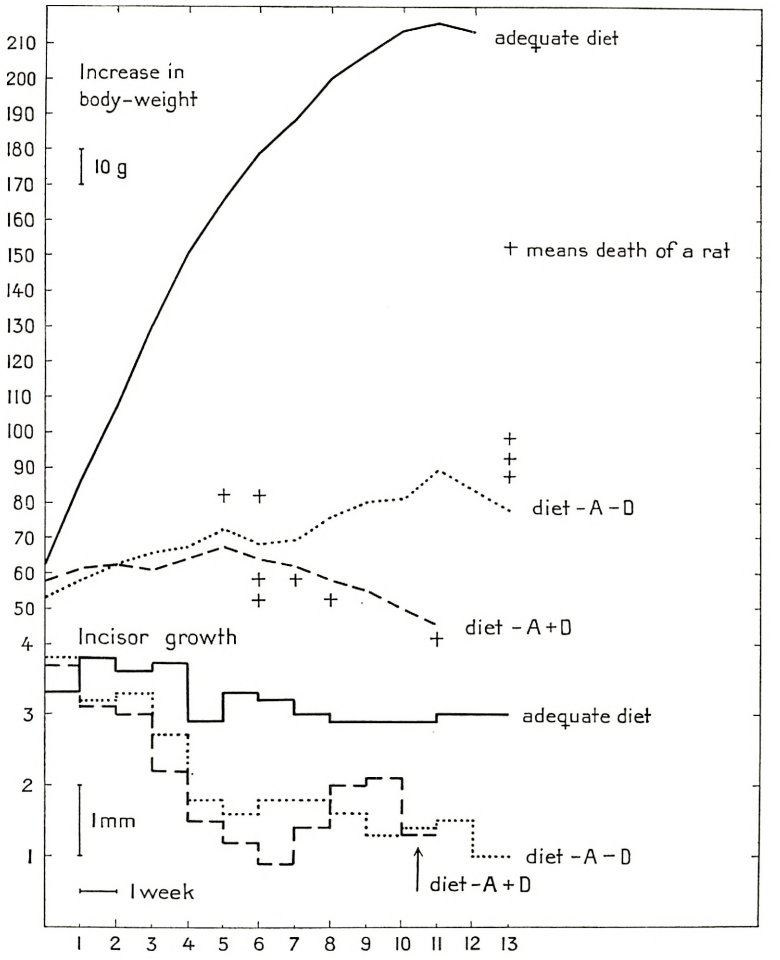
The adequate diet was the same as that used in Series I. The rate of growth of the incisors and the increase in body weight was measured in nine young rats belonging to two litters, 35 and 37 days old at the beginning of the experiment. The measurements were continued for 13 weeks, being made weekly for the first 8 weeks and twice during the last five.

At the outset, the young rats weighed 47—75 grm., on an average, 62 grm.; at the end, their weight was 138—272 grm. The average weekly rate of growth of their incisors was 3.35 mm., with a standard deviation of 0.47 mm. for a single measurement (see Table I). This standard deviation is considerably larger than that in Series I. As the rate of tooth growth is found to be larger in this series on young rats than in Series I on adult rats (3.35 mm. weekly as against 2.69 mm. weekly), the question as to whether the rate of tooth growth has decreased during the experiment with young rats must be gone into. This would explain why the standard deviation is larger in Series II than in Series I.

Table II. Extrusive growth per week in mm, of the incisors of young rats, fed on different diets.

Week no.	1	2	3	4	5	6	7	8	9	10	11	12	13
		3.6			3.3			3.0			3.0		
9 young rats fed on an adequate diet	3.3	3.8	3.6	3.7	2.9	3.3	3.2	3.0	2.9	2.9	2.9	3.1	3.1
5 — — - - - a diet — A + D	3.7	3.1	3.0	2.2	1.5	1.2	0.9	1.4	2.0	2.1	1.3		
5 — — - - - — — A — D	3.8	3.2	3.3	2.7	1.8	1.6	1.8	1.8	1.6	1.3	1.4	1.5	1.0
5 — — - - - — — B	3.3	3.4	3.1	3.1	2.9	2.0	2.5	2.4	1.8	1.5	1.9	1.8	

Curve A and Table II show that the average rate of growth of the incisors of the young rats has decreased



Curves A. Average increase in body weight and average weekly extrusive incisor-growth in young rats fed on an adequate diet and on diets deficient in vitamin A and vitamin A and D respectively.

during the course of the experiment. This can easily be seen if the first 12 weeks of the experiment are divided into 4 periods, each of 3 weeks. In these 4 periods the average weekly rates of growth of the incisors are respectively, 3.6 mm., 3.3 mm., 3.0 mm., 3.0 mm. Each period

represents 27 measurements and the standard deviation of its average is $\frac{0.47}{\sqrt{27}} = 0.09$. The decrease in the rates is, therefore, significant.

This decrease in the rate of growth of the incisors as the young rat grows older must be taken into consideration in the following experiments.

The rate of tooth growth in quickly growing young rats is greater than in those that grow more slowly. This is seen in Table III.

Table III.

	Average increase in weight per rat in 8 weeks	Average weekly rate of growth of incisors
Group 1. 3 rats	101 grm.	3.25 mm.
— 2. 3 —	148 —	3.38 —
— 3. 3 —	166 —	3.43 —

In experiments on young rats fed on other adequate diets a still more rapid growth of the incisors has been observed in this laboratory.

Series III. Young rats on diets deficient in vitamin A.

The diet was the same as that in Series I and II but with 15 % oxidised lard instead of 15 % butter fat. This diet is practically devoid of vitamin A and vitamin D. From the investigations of MAY MELLANBY (1928), and others, it is known that a deficiency of vitamin D influences the structure of growing teeth. We have therefore measured the effect of diets devoid of vitamin A and vitamin D (called: Diet $-A\ -D$) as well as those only devoid of vitamin A (called: Diet $-A\ +D$). Rats on the

diet —A +D received 5 drops of a pharmaceutical solution of irradiated ergosterol daily.

The rats used in all the following experiments are of the same initial age (5 weeks) as those in Series II.

Series III A. Diet —A +D. Five young rats weighing 55—62 grm. (average, 58 grm.) were used. Two died after 6 weeks, the other three after 7, 8, and 11 weeks, respectively. The average increase in weight is shown in Curves A, the average weekly growth of the incisors in Curves A and Table II.

Series III B. Diet —A —D. Five young rats weighing 45—56 grm. (average, 53 grm.) died after 5, 6, and 14 weeks, see Curves A and Table II.

Some of these rats lived longer than is usual in experiments with vitamin A free diets.

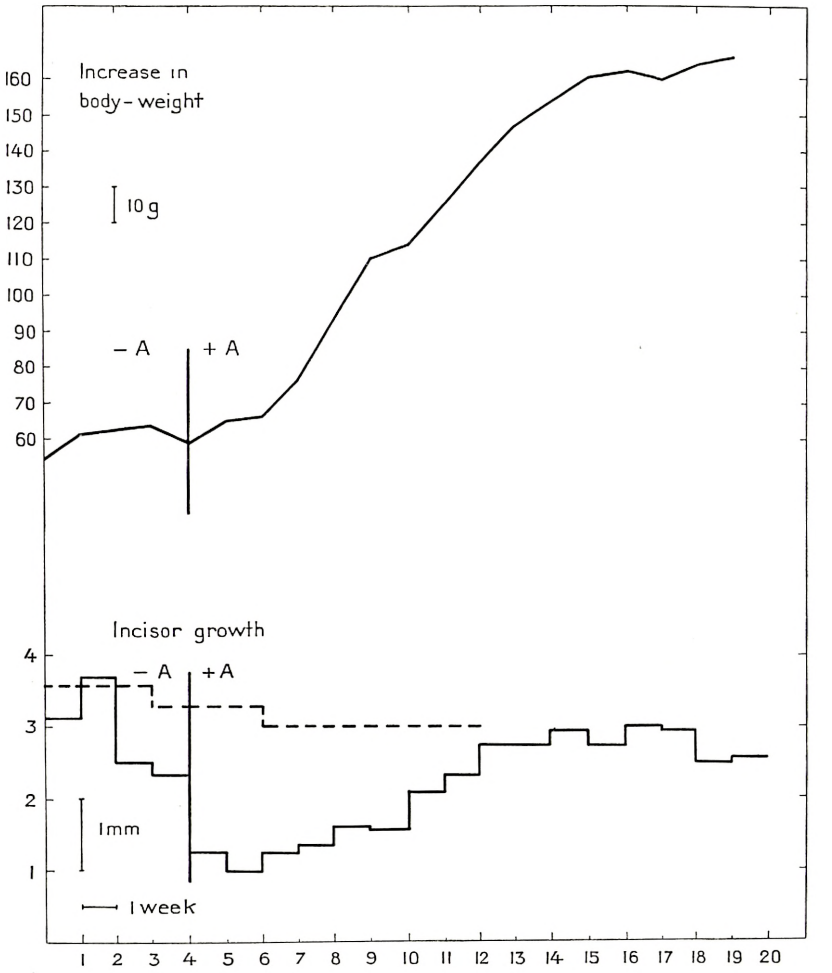
Curves A and Table II show that the weekly rate of growth of the incisors decreased from the fourth experimental week in both series. In the fourth week the average rate of growth of the incisors was 2.2 mm. in Series III A, and 2.7 mm. in Series III B, as against 3.7 mm. in young rats of the same age which had been adequately nourished. In the following weeks the rate of growth of the incisors further decreased to about 1.3—2.1 mm. weekly in the young rats on deficient diets. The difference between the weekly rate of growth of the incisors in the adequately nourished animals and in those nourished deficiently is 1.1 mm.—2.3 mm. from the 5th week and on. The standard deviation in the average measurements of five rats is less than $\frac{0.47}{\sqrt{5}}$ or 0.2 mm. This difference is therefore significant.

The retardation in the growth of the incisors is the same in Series III A as in Series III B. This means that the deficiency of vitamin A and the combined deficiency of vitamins A and D produce identical effects on this process. The growth of the incisor continues in young rats deprived of vitamin A in their food when the increase in weight has stopped and goes on until the death of the animals, but only at about half the normal rate.

Series III C. This experiment was performed in the same way as vitamin A estimations by the curative method, in order to see if the growth of the incisors could be restored by giving vitamin A again after having first been restrained by a deficiency of this vitamin.

Three young rats (weight 52—59 grm.) were fed on diet —A —D. In the fourth experimental week all three decreased in weight and exhibited incipient xerophthalmia. The average weekly rate of growth of the incisors decreased from 3.1—3.7 mm. to 2.4 mm. (see Curves B). Then a daily supplement of 0.2 grm. cod-liver oil was given. The avitaminotic symptoms disappeared and the body weight increased. But the weekly rate of growth of the incisors decreased still further during the first two weeks when cod-liver oil was given and reached the low value of 1.0 mm. The experiment was continued for 20 weeks. At the end, the rats weighed 155, 168, and 199 grm., and the growth of their incisors had regained an almost normal average weekly rate of 2.9—3.0 mm.

In this experiment an after-effect of the vitamin A deficiency was observed on the growth of the incisors. While a supply of cod-liver oil immediately cured the sore eyes and produced an increase in body weight in the A avita-



Curves B. Average increase in body-weight and average weekly extrusive incisor growth in young rats, fed during four weeks on a diet deficient in vitamin A, and then on an adequate diet (compared with the average weekly incisor growth in young rats, fed on an adequate diet all the time, shown in three-weekly periods).

minotic rats, the rate of the growth of their incisors further slowed down for two weeks after the supply of vitamin A had been resumed, after which restoration set in.

Series III D. A preliminary experiment was carried out on the effect of different suboptimal doses of vitamin A on the rate of growth of the incisors. Three groups, each of two rats, were used. The initial weight of the rats was 40—68 grm. From the beginning of the experiment the three groups received a daily supplement to the vitamin A free basal diet of 0.25 mg., 0.50 mg., and 1.00 mg., respectively, cod-liver oil (containing about 1500 internat. vitamin A units per grm.). In Table IV the average weekly rate of growth of the incisors for periods comprising three weeks each is tabulated for the three groups, and compared with the corresponding rates in young rats on an adequate diet (Series II), and on a diet devoid of vitamin A.

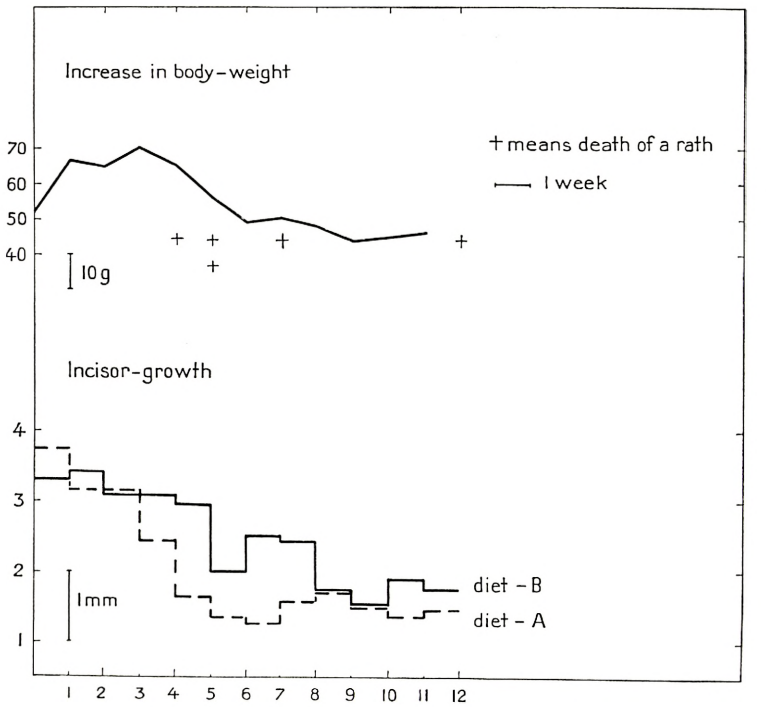
Table IV. Rate of growth of the incisors of young rats receiving different daily amounts of vitamin A.

	Adequate diet	Cod-liver oil per day			Diet devoid of vitamin A
		1.00 mg.	0.50 mg.	0.25 mg.	
1st— 3rd week..	3.6 mm.	3.5 mm.	3.5 mm.	3.3 mm.	3.3 mm.
4th— 6th -- ..	3.3 -	2.9 -	2.9 -	2.6 -	1.8 -
7th— 9th — ..	3.0 -	2.2 -	1.8 -	1.6 -	1.6 -
10th—12th — ..	3.0 -	2.0 -	1.5 -	1.2 -	1.4 -

The results are only provisional because each group comprised only two rats. It is seen that the depressing influence of the smaller doses on the rate of tooth growth is not apparent before the 7th—9th week of the defective feeding. Still, the results suggest the possibility of using measurements of the rate of growth of the incisors as a biological method for the quantitative estimation of vitamin A.

Series IV. Young rats on a diet deficient in the vitamin B complex.

In the preceding experiments deficiency of vitamin A was found to exert a depressing influence on the rate of



Curves C. Average increase in body-weight and average weekly extrusive incisor growth in young rats fed on a diet deficient in the vitamin B complex (compared with the average weekly incisor growth in young rats fed on a diet deficient in vitamin A).

growth of the incisors. In order to see whether this influence is specific to vitamin A deficiency, or is also found in other dietary deficiencies, an experiment with a diet devoid of the vitamin B complex was made.

This diet was the adequate food mixture used in Series I and II, but without the 5 % dried autolyzed yeast. The

casein was especially purified from content of the vitamin B complex.

Five young rats, weighing 44—58 grm. (average, 52 grm.) were used. Their body weight decreased after a few weeks and they died after 4, 5, 5, 7, and 12 weeks respectively. The average weight curve is seen in Curves C, and the average weekly rates of growth of the incisors in Curves C and Table II.

The curves and the table show that the weekly rate of growth of the incisors decreases from the 6th week of the experiment, being 2.0—2.5 mm. from the 6th to the 8th, and 1.5—1.9 mm. from the 9th to the 12th week, as against 3.0—3.3 mm. in young rats on an adequate diet. The decrease in the rate occurs later in this experiment than in the experiments concerning vitamin A deficiency (in the 6th week when the vitamin B complex is deficient, as against the 4th week when vitamin A is deficient).

Discussion.

A considerable decrease in the rate of growth of the incisor teeth of young rats fed on a diet devoid of vitamin A was found in Series III. This result agrees with the histological investigations of S. E. Wolbach and P. R. Howe (1933) who found an atrophy of the enamel organ followed by atrophy of the odontoblasts of the incisors of rats fed on a diet deficient in vitamins A and D. The atrophy finally involved the whole length of the enamel organ, including the basal formative end.

Series III A compared with Series III B shows that the presence or absence of vitamin D in a diet devoid of vitamin A is without influence upon the decrease in the rate of growth of the incisors effected by vitamin A deficiency.

The pushing out of the tooth, accordingly, seems to be dependent on processes not influenced by vitamin D.

An after-effect of vitamin A deficiency upon the rate of growth of the teeth is found in Series III C. The rate of growth is nearly normal again only about three months after the rat has again been given a sufficient supply of vitamin A, although the morphological re-establishment of the enamel organ is completed 19 days after vitamin A is given again (Wolbach and Howe (1933)). C. E. Bloch (1931) found no after-effect from severe vitamin A deficiency on the teeth of human beings.

Orr and Richards (1934) raised the problem as to whether vitamin A deficiency has a direct influence on the growth of bony structures or not. The experiments described in this paper cannot settle this question. A correlation between vitamin A deficiency and retardation of the rate of growth of the extrusive incisor teeth of rats has been proved, but the mechanism connecting these two phenomena has not been investigated. Wolbach and Howe (1933) described changes in the enamel organ of rats and guinea-pigs fed on a diet deficient in vitamin A, and regarded these changes as one of the primary consequences of vitamin A deficiency common to many epithelial organs. The changes in the enamel organ may be one of the factors retarding the growth of the tooth.

Deficiency in vitamin A is not the only dietary deficiency which is able to retard the rate of growth of the incisors of rats. In Series IV, deficiency of the vitamin B complex was found to have the same effect, though it was of later occurrence. Dalldorf and Fall (1930) observed the same effect in experiments on guinea-pigs fed on a diet deficient in vitamin C.

Summary.

1. The rate of extrusive growth of the incisor teeth is, on an average, 2.7 mm. per week in adult rats, and 3.3 mm. per week in young rats, fed on an adequate diet. The standard deviation for the single measurement was 0.30 mm. for adult, and 0.47 for young rats.

2. In young rats on a diet deficient in vitamin A, this rate decreases after three weeks to about 2.5 mm. and in the following weeks to about 1.5 mm.

3. It makes no difference to this effect whether the diet which is deficient in vitamin A contains vitamin D or not.

4. Different daily suboptimal amounts of vitamin A, when fed for periods of longer than 6 weeks, retard the rate of extrusive growth of the incisor teeth to different degrees.

5. When a sufficient supply of vitamin A is given to young rats, the rate of growth of whose incisors has been retarded by deficiency of vitamin A, an after-effect appears from the deficiency, the rate decreasing further during the first two weeks after the supply of vitamin A is resumed, in spite of the immediate effect of the supply on other avitaminotic symptoms in the animals. The rate of growth of the incisors is about normal again only after three months' adequate feeding.

6. A diet deficient in the vitamin B complex also depresses the rate of extrusive growth of the incisors of young rats, but the effect occurs later than in vitamin A deficiency.

From the University Institute of Hygiene, Copenhagen.

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